Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 66 (2007) 210–219
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

# Synchronized and sustained release of multiple components in silymarin from erodible glyceryl monostearate matrix system

Cheng Lu [a], Yi Lu [a,*], Jian Chen [a], Wentong Zhang [b], Wei Wu [a,*]

[a] School of Pharmacy, Fudan University, Shanghai, PR China
[b] School of Public Health, Fudan University, Shanghai, PR China

Received 20 September 2006; accepted in revised form 13 November 2006
Available online 22 November 2006

**Abstract**

Development of sustained delivery systems for herbal medicines was very difficult because of their complexity in composition. The concept of synchronized release from sustained release systems, which is characterized by release of multiple components in their original ratio that defines a herbal medicine, served as the basis for keeping the original pharmacological activity. In this study, erodible matrix systems based on glyceryl monostearate and polyethylene glycol 6000 or poloxamer 188 were prepared to perform strict control on synchronized release of the five active components of silymarin, i.e. taxifolin, silychrystin, silydianin, isosilybin and silybin. The matrix system was prepared by a melt fusion method. Synchronized release was achieved with high similarity factor $f_2$ values between each two of the five components. Erosion profiles of the matrix were in good correlation with release profiles of the five components, showing erosion-controlled release mechanisms. Through tuning some of the formulation variables, the system can be adjusted for synchronized and sustained release of silymarin for oral administration. In vitro hemolysis study indicated that the synchronized release samples showed a much better stabilizing effect on erythrocyte membrane.


*Keywords:* Synchronized release; Sustained release; Glyceryl monostearate; Polyethylene glycol 6000; Poloxamer 188; Silymarin; Erosion

## 1. Introduction

Herbal medicines have been used for thousands of years, and nowadays we see a steady rise in the number of patients and medical practitioners who prefer to use herbal medicines as a supplement to or substitute for prescription drugs. However, there is little progress in the development of dosage forms of herbal medicines due to complexity of their constituents. Although several novel dosage forms originally designed for chemical drugs have also been applied for herbal medicines, sustained or controlled release drug delivery system has not yet been investigated profoundly. When developing such complicated systems of herbal medicines, questions arise: "To what extent and how shall we control the release rate of each individual component as herbal medicines are undoubtedly composed of multiple components which may differ greatly in activity and physicochemical properties?".

To answer this question, it is necessary to understand what defines an herbal medicine first. The pharmacological activity of an herbal medicine depends on the overall activity of a variety of active components. Synergistic action of these components is the basis for herbal recipes [1]. In other words, each active component plays an important role in the interconnected system. Leaving out a single component or changing the relative ratio of their components may result in a change in pharmacological action. So, an herbal

*Abbreviations:* TF, taxifolin; SC, silychristin; SD, silydianin; ISB, isosilybin; SB, silybin; GMS, glyceryl monostearate; PEG, polyethylene glycol; PXM, poloxamer; HPMC, hydroxypropyl methylcellulose; SDS, sodium dodecyl sulfate.

* Corresponding authors. Department of Pharmaceutics, School of Pharmacy, Fudan University, 138 Yi Xue Yuan Rd., Shanghai 200032, PR China. Tel.: +86 21 5423 7833; fax: +86 21 6417 0921 (W. Wu, Y. Lu).
*E-mail address:* wuwei@shmu.edu.cn (W. Wu).

0939-6411/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2006.11.008

medicine may be defined as a collection of active components in relatively steady ratios. The definition serves as the basis for quality control of herbal medicines. The recently enacted regulation on control of fingerprint components of herbal medicines is evidence of the pervasive awareness of the importance of component ratios [2–4], although it leaves much to be desired to find the optimum ratios. A functionally sustained or controlled release system of herbal medicines should not change and should control to a narrow range the ratio of the components, which may have been optimized through pharmacological screening. It means that the active components should be released in their original ratio, i.e. synchronized release.

One may argue that although some of the orally administered herbal medicines were embodied with fixed component ratio, they may undergo a process of dissolution before arrival at the absorption site. This seemed to serve as one reason to deny the basis of controlling of the original ratios, since once the dissolution begins, components with different physicochemical properties might dissolve in different rates and release the 'drug' in altered component ratios. It seemed that for such herbal medicines, the original component ratio virtually does not play a leading role in the pharmacological activity of the drug. However, inspecting the developing process of an herbal medicine, it was found that herbal medicines were actually expected to dissolve simultaneously at the absorption site. In fact, the pharmacological activity of a herbal medicine was primarily set up using its solution form, not to mention hundreds of traditional Chinese medicines, which is routinely boiled into solutions before oral administration. Furthermore, efforts have been made to enhance the dissolution of water-insoluble herbal medicines, hoping that they may be administered in a dosage form that is more like a solution [5]. So, the original ratio of multiple components was the determinant factor of an herbal medicine, and the concept of synchronized release was not without basis.

However, to achieve synchronized release seems to be a difficult task, because the active components may differ significantly in chemical structure and physicochemical properties, which will further result in divergence of their release profiles whenever diffusion contributes much to the release mechanisms. To achieve synchronous release, the release rate of each individual component might not depend on their structure. In other words, efforts must be made to restrict the contribution of diffusion mechanisms to drug release. Fig. 1 provides a simple interpretation of the underlying mechanisms to achieve synchronous release in this study. Suppose the 'herbal medicine' is a block of homogeneously mixed components. The block of herbal medicine may be divided into infinite pieces of small doses, each of which retains the properties of the mother herbal medicine. Sustained release of herbal medicine can be achieved by continuous release of the infinite small doses, keeping the original ratio of the active components throughout the release process.

Although synchronized release is still a relatively new concept, several strategies to develop sustained or controlled release of herbal medicine do apply such concept. Song et al. [6] developed a multi-unit system of traditional Chinese medicine (heart-protecting mask) pH-dependent gradient pellets. This system was made up of three kinds of Eudragit polymer-coated pellets that can dissolve and release the active components at pHs of 5.5, 6.2 and 6.8, respectively. The release profiles of the active components were typical of three-pulse pulsatile release, and those of borneol and total ginsenoside were compared and regarded as 'synchronous'. Multiple-pulse design seems to make this system perfect, but implausible industrially. To prevent the contribution of diffusion to release, attention was paid to erodible systems from which drug release was only a function of the erosion rate of the matrix. Baluom et al. [7] reported an erodible matrix tablet system that is capable of releasing sulpiride synchronously with P-gp inhibitors, verapamil or quinine, and improved absorption of sulpiride was observed.

Herein, we proposed a simple approach to achieve sustained and synchronized release of the multiple components of silymarin from an erodible matrix system, which is made up of GMS and PEG or PXM. GMS, a weak surfactant of high hydrophobicity [8], has been formulated into matrix system to retard drug release [9–12]. Drug release from GMS-based matrix system was predominantly erosion- controlled, especially for less water-soluble entities [10]. As drug release from GMS system was much slower for several days, water-soluble carriers like PEG or poloxamer were incorporated to tune the release rate to meet the demands of oral administration. A heat fusion method [13,14] was employed to prepare silymarin GMS/PEG (or PXM) matrix system, which is virtually a solid dispersion with each of the active components of silymarin dispersed homogeneously throughout. Silymarin is a well-known heptaprotector and is used to treat a variety of liver disorders, including acute and chronic viral hepatitis, toxin- and drug-induced hepatitis and cirrhosis, and alcoholic liver disease [15]. It is extracted from *Silybum marianum* L.Gaertn (milk thistle) and is, composed of mainly five active components i.e. TF, SC, SD, SB and ISB (Fig. 2) [16,17], which make up about 60% flavnoligans of silymarin [17]. The physicochemical properties of these components differ greatly as a result of their structural difference. The goal of this study was to achieve synchronous release of these active components.

## 2. Materials and methods

### 2.1. Materials

Silymarin (59.8% total flavonolignans by HPLC) was purchased from Pan-jin-hua-cheng Pharm. Co., Ltd. (Liaoning, China). Methanol and 1,4-dioxane were of HPLC grade (Tedia, USA). Taxifolin was purchased from Fluka (USA). Silybin standard was purchased from National

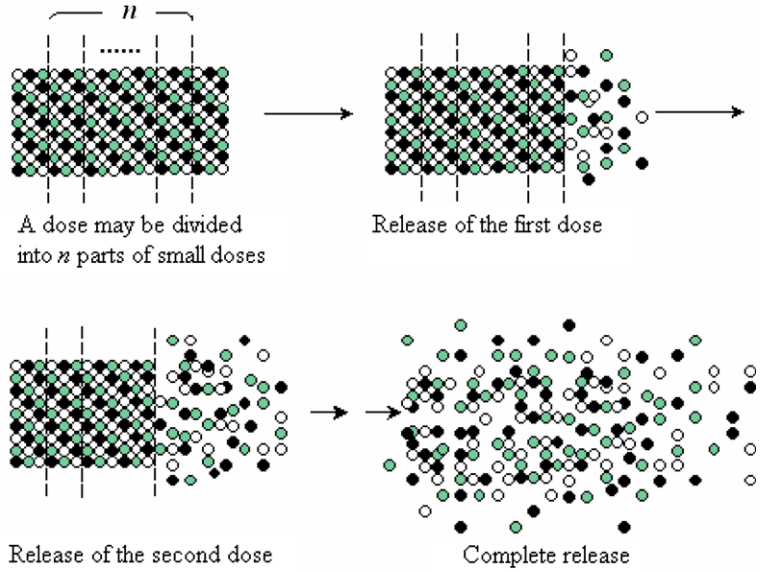


Fig. 1. Schematic illustration of the mechanism of synchronized and sustained release of herbal medicines (Note that in real systems release may take place in all directions).

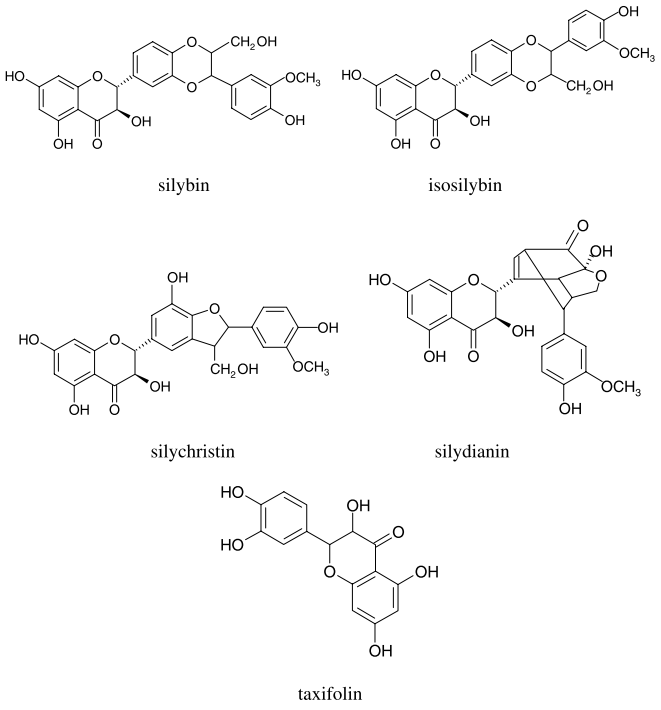


Fig. 2. Chemical structure of the five active components of silymarin.

Institute for the Control of Pharmaceutical and Biological Products. Silychristin, isosilybin and silydianin were kindly gifted by Shanghai Institute for Drug Control. HPMC K4M was a Dow Chemicals product and kindly gifted by Shanghai Colorcon Coating Technology Ltd., PEG 6000, PXM 188 and GMS were of pharmaceutical grade and were purchased from local distributors. Other reagents were of analytical grade.

### 2.2. Preparation of silymarin HPMC matrix tablets

Matrix tablet based on HPMC is a well-accepted sustained release delivery system for chemical drugs. Here, silymarin HPMC matrix tablet was firstly prepared by a direct compression method and evaluated for in vitro release. Powders of silymarin and the excipients were mixed homogeneously by a mortar and pestle. Then, the powdered mixture was directly compressed into flat cylindrical tablets 6 mm in diameter by a ZDY-8 model single-punch compressor (Far East Pharmaceutical Machinery Ltd., Shanghai, China). Each silymarin/HPMC matrix tablet contained 50 mg of silymarin, 40 mg of HPMC K4M, 25 mg of lactose monohydrate and 1.5 mg of magnesium stearate. The batch size was 100 tablets.

### 2.3. Preparation of silymarin GMS/PEG (or poloxamer) matrix system

The matrix type solid dispersions of silymarin and GMS/PEG (or PXM) were prepared by a melt fusion method. Firstly, PEG 6000 or PXM was melted at about 90 °C. Silymarin was added into this melted mixture under continuous stirring until a homogeneous solution was obtained. Then GMS was added, melted and stirred for 5 min to achieve a homogeneous mixture, which was settled for a few minutes to release bubbles. The melt mixture was poured into a stainless steel cylindrical mold (∅1 × 1 cm), which was cooled previously to −18 °C. After that, the mold together with the melt mixture was stored under −18 °C for 4 h to congeal. After removal from the mold, the cylindrical matrix was further stored for 18 h in a desiccator at ambient temperature. If not specified, the drug/carrier ratio was set to 1/20, and the total weight of the final matrix was about 1.0 g.

### 2.4. Determination of the active components of silymarin by HPLC

An HPLC/UV method [16] was employed to determine the five active components of silymarin, i.e. TF, SC, SD, ISB and SB. The Agilent 1100 series HPLC system (Agilent, USA) was composed of a quaternary pump, a degasser, an autosampler, a column heater, and a tunable ultraviolet detector. Separation of the five active components was performed on a C18 column (Kromasil, 5 μm, 4.6 × 250 mm, Sweden) guarded with a refillable precolumn (C18, 2.0 × 20 mm, Alltech, USA), and detected at 288 nm. The mobile phase consisted of methanol (A) and aqueous dioxane (B) (90% water + 10% dioxane) with gradient elution (Fig. 3). The flow rate was fixed to 1 ml/min, and the column temperature was set to 40 °C.

A total elution time of 40 min was sufficient to separate the five active components with high resolution (Fig. 4). Calibration curve (peak area $A$ versus concentration $C$), linear range, limit of quantification and regression coefficient are given in Table 1. The assay accuracies of the five components in all of the release media were between 99.76% and 102.1%, and within-day and between-day precision were 0.45–1.03% and 0.57–1.85%, respectively.

### 2.5. Release studies

The release studies were carried out based on the Chinese Pharmacopoeia (2005 Ed.) paddle method. Release medium was 900 ml of distilled water (pH 6.5) thermostatically maintained at 37 ± 0.5 °C and stirred at 75 rpm. Under such conditions, solubility of each of the five components was enough to maintain sink conditions. Release in pH 1.2 hydrochloride solution and pH 4.0 acetate buffer was also studied. Stability study showed that the five components were stable in all of the release media for at least 24 h. At predetermined time intervals, 5 ml of release medium was withdrawn and filtered through 0.45 μm Nylon film (Peninsula Trading Ltd., Shanghai, China). The filtrate was assayed for TF, SC, SD, ISB and SB. Meanwhile, 5 ml of fresh release medium was supplemented to keep constant volume.

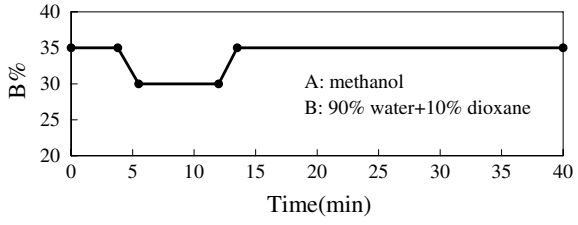


Fig. 3. Gradient elution program for the determination of multiple components of silymarin by HPLC/UV.

The release data were presented as means of three determinations. The similarity factor $f_2$ [18–21] was introduced to justify the synchronism of each two of the release profiles. $f_2$ was defined as:

$$f_2 = 50 \log \left\{ \left[ 1 + \frac{1}{m} \sum_{j=1}^{m} W_j |R_j - T_j|^2 \right]^{-0.5} \times 100 \right\}$$

where $R_j$ and $T_j$ are cumulative drug released at measuring time of the reference and test products, respectively; $m$ is the total number of sampling time points; $W_j$ is an optional weight factor. The similarity factor fits the result between 0 and 100. It is 100 when the two profiles are identical and approaches 0 as dissimilarity increases. Values of over 50 were accepted as 'similar' [19].

### 2.6. Erosion and hydration studies

Erosion and hydration of the matrix tablets was evaluated using a release study assembly [21,22]. Samples were collected after a definite time of erosion and dried for 48 h under vacuum at ambient temperature to constant weight. The erosion and relative water uptake percentage was calculated as:

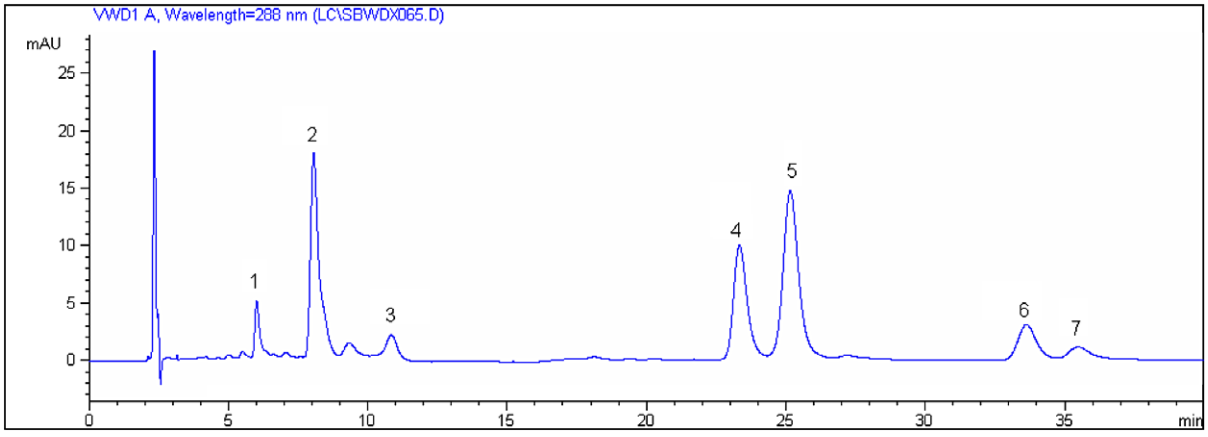


Fig. 4. Chromatogram of the multiple components of silymarin separated by HPLC/UV. 1, taxifolin; 2, silychristin; 3, silydianin; 4 and 5, silybin diastereomers and 6 and 7, isosilybin diastereomers.

Table 1
Calibration curve, regression coefficient, linear range and limit of quantification of taxifolin, silychristin, silydianin, isosilybin and silybin determined by HPLC/UV

| Component | Calibration curve | Regression coefficient | Linear range (μg/ml) | Limit of quantification (μg/ml) |
|---|---|---|---|---|
| Taxifolin | $A = 70.292C–0.0974$ | 0.9999 | 0.01702–11.35 | 0.01702 |
| Silychristin | $A = 65.739C–3.2111$ | 0.9999 | 0.03495–23.30 | 0.03495 |
| Silydianin | $A = 39.656C–2.0403$ | 0.9999 | 0.03683–24.55 | 0.03683 |
| Isosilybin | $A = 44.246C–3.5715$ | 0.9998 | 0.04095–27.30 | 0.04095 |
| Silybin | $A = 45.884C–1.1869$ | 0.9999 | 0.02265–67.95 | 0.02265 |

$$\text{erosion } (\%) = \frac{W_t - W_0}{W_0} \times 100\%$$

$$\text{water uptake } (\%) = \frac{W_{\text{wet}} - W_t}{W_t} \times 100\%$$

where $W_0$ is the initial weight of the matrix, $W_{\text{wet}}$ is the wet weight of the matrix at time $t$, and $W_t$ is the dry weight of the matrix at time $t$.

### 2.7. Erythrocyte membrane-protecting studies

Silymarin has been used clinically for the treatment of liver diseases. Although the protective mechanism has not been elucidated completely, it is generally accepted that the flavonoids exert a membrane stabilizing action [15]. Here, we carried out a preliminary study on the cytoprotective properties of synchronized release of silymarin on erythrocyte hemolysis [23,24].

Blood was collected from a healthy adult rabbit by cardiac puncture into heparinized tubes and centrifuged at 2000*g* for 10 min at ambient temperature. The plasma and buff coat were removed, and packed erythrocytes were washed three times with five volumes of isotonic NaCl solution. A final 2% erythrocyte suspension was obtained by suspending in isotonic NaCl solution.

Synchronized release samples were withdrawn intermittently during the release test process of GMS/PEG 6000 synchronized release matrix. The non-synchronized release samples were obtained through collection at different time intervals during the release process of silymarin powder. To facilitate the hemolysis study, the release test was carried out in isotonic NaCl solution. At certain time intervals, release samples were collected, filtered and assayed as described above for TF, SC, SD, SB and ISB.

The hemolysis test was performed firstly using distilled water as shocking agent [23]. To a 5 ml centrifuge tube containing 1 ml of 2% erythrocyte suspension, 1 ml of either synchronized or non-synchronized release sample was added. After mixing by slight shaking, distilled water was added, and appropriate amount of isotonic NaCl solution was supplemented to make a total volume of 4 ml. The mixture was incubated at 37 °C for 1 h, and then centrifuged at 2000*g* for 10 min. Absorbance of the supernatant was determined at 540 nm by a Spectrumlab 54 UV spectrophotometer (Shanghai Lengguang Technologies Ltd., China). Absorbance at 100% hemolysis ($A_{100\%}$) was determined by adding 3 ml of distilled water to 1 ml of 2% erythrocyte suspension, and control absorbance of autolysis ($A_{\text{auto}}$) was determined by adding 1 ml of dissolution sample and 2 ml of isotonic NaCl solution (without shocking agents). The hemolysis percentage was calculated as:

$$\text{hemolysis } (\%) = \frac{A_{\text{sample}} - A_{\text{auto}}}{A_{100\%} - A_{\text{auto}}} \times 100\%$$

All the measurements were in triplicate, and the volume of total shocking agent for 50% hemolysis ($H_{50}$) with 95% confidence limits was calculated by SPSS 11.0 software (SPSS Inc., Chicago, USA). For convenience, $H_{50}$ for distilled water as shocking agent was expressed as the concentration (w/v) of NaCl in the system [23].

The hemolysis test was also performed using 0.01% SDS as shocking agent [25], following similar procedures replacing distilled water with 0.01% SDS. To obviate possible effect of hypotonicity, SDS was prepared in isotonic NaCl solution.

## 3. Results and discussion

Fig. 5 shows the release profiles of TF, SC, SD, ISB and SB from an HPMC K4M matrix tablet. It is evident that the release profiles of these five components did diverge gradually. At a time of 12 h, release of TF approached 42.95%, which is the fastest among the five components, while that of SC, SD, SB and ISB was in decreasing order of about 27.72%, 23.77%, 20.13% and 8.11%, respectively. The original content of TF, SC, SD, ISB and SB in

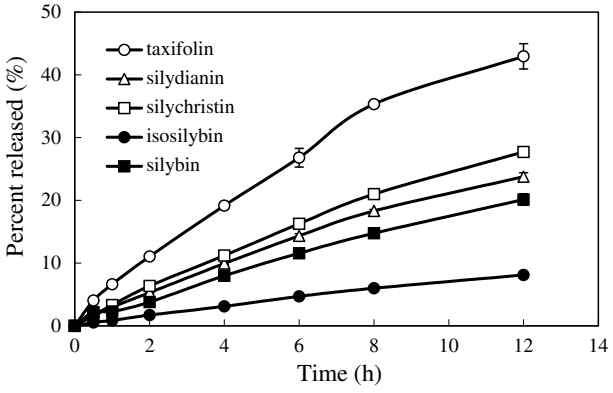


Fig. 5. Release profiles of multiple components of silymarin from HPMC matrix tablets.

silymarin determined by HPLC was 1.67%, 12.91%, 3.51%, 8.35% and 33.40%, respectively. Setting that of SB to 1, the ratio of the five components, TF/SC/SD/ISB/SB, can be presented as 0.05/0.39/0.11/0.25/1. Once the release began, the original ratio underwent significant change (Table 2). As the release process continued, the ratios of the five components released kept changing. It was obvious that a common HPMC-based sustained release system could not achieve synchronized release of the investigated components. Difference in dissolution rate and diffusivity of the five components might contribute to this result. As the variation in ratio of the five components was great, the drug 'silymarin' that defined by the original ratio did not exist anymore. So, change in its pharmacological effect was unavoidable, which was not desirable as strict control on drug quality and pharmacological effect was the ultimate goal of drug development.

Fig. 6 shows the release profiles of TF, SD, SC, ISB and SB from GMS/PEG6000 (15/85) cylindrical matrix at drug/carrier ratio of 1/20 in distilled water. Synchronized release was achieved for a time duration of at least 12 h as expected. $f_2$ values of each two of the release profiles and their arithmetic mean are given in Tables 3 and 4. $f_2$ values were all over 50 at GMS/PEG6000 or GMS/PXM ratios from 5/95 to 40/60. It is indicated that release of the five components was 'similar' under the experimental conditions in this study. There was excellent similarity between SB and ISB with $f_2$ values of over 90 both for GMS/PEG6000 and GMS/PXM systems. There was also good similarity between SC release and SB or ISB release with $f_2$ values of over 80. For TF and SD, similarity was not as good as that for SC, SB and ISB, especially at lower GMS/PEG6000 ratios. However, when GMS/PEG6000 ratio increased to over 15/85, $f_2$ values of about 80 were observed, showing GMS-dependent increase in similarity. $f_2$ values of each two of the release profiles of TF/SD and TF/SC were much less than those between TF/ISB, TF/SB, SD/SC, SD/ISB and SD/SC. Only limited increase in $f_2$ values was observed for GMS/PXM system as GMS weight increased. ANOVA was performed to evaluate the difference between GMS/PEG6000 and GMS/PXM 188 systems, and results showed that there was significant difference ($P < 0.0001$) in $f_2$ values. GMS/PEG6000 system seemed to result in better synchronism than GMS/PXM 188 system.

Table 2
Dynamic changing of the ratio of taxifolin/silydianin/silychristin/isosilybin /silybin after releasing from silymarin HPMC K4M matrix tablet

| Time (h) | Taxifolin | Silychristin | Silydianin | Isosilybin | Silybin |
|---|---|---|---|---|---|
| Original | 0.05 | 0.39 | 0.11 | 0.25 | 1 |
| 0.5 | 0.37 | 1.12 | 0.32 | 1.00 | 1 |
| 1 | 0.38 | 1.32 | 0.40 | 0.66 | 1 |
| 2 | 0.32 | 1.19 | 0.39 | 0.55 | 1 |
| 4 | 0.31 | 1.24 | 0.38 | 0.64 | 1 |
| 6 | 0.28 | 1.18 | 0.36 | 0.61 | 1 |
| 8 | 0.30 | 1.18 | 0.37 | 0.62 | 1 |
| 12 | 0.26 | 1.13 | 0.36 | 0.62 | 1 |

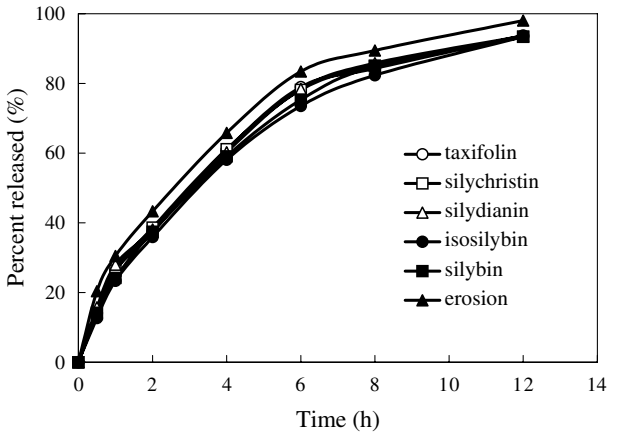


Fig. 6. Release profiles of multiple components of silymarin from and erosion of GMS/PEG6000 (15/85) matrix system with a drug/carrier ratio of 1/20 in distilled water.

Although similarity in the release profiles of TF, SC, SD, ISB and SB was observed, there was disparity in $f_2$ values. This means that the chemical structure has, more or less, an effect on release synchronism. Since ISB and SB are isomers with identical moieties, their release profiles are almost identical. Similarity in chemical structure of SC and ISB or SB was also observed, and good synchronism exists between their release profiles. As for TF and SD, difference in chemical structure compromises their synchronism with SC, ISB and SB, although their release profiles can still be regarded as 'similar' with $f_2$ values greater than 60.

As PEG6000 and PXM were highly water-soluble carriers, increase in their content in the matrix led to increase in release rate. As shown in Table 5, when GMS/PEG6000 was 40/60, percentage of drug released at 12 h ($P_{12}$) was 40.87%, while at GMS/PEG 6000 ratio of 5/95, the $P_{12}$ was 92.21%. This served as the basis for the formulation of sustained and synchronized release delivery systems of herbal medicines, because the overall release rate can be tailored for oral administration, adjusting the ratio of GMS to water-soluble carriers without changing much the similarity of the release profiles.

In order to evaluate the effect of release medium pH, release of GMS/PEG6000 (15/85) matrix system in a drug/carrier ratio of 1/20 was also evaluated in pH 1.2 HCl solution and pH 4.0 acetate buffer. As shown in Figs. 7 and 8, synchronized release of TF, SC, SD, ISB and SB was also achieved in these two release media. The fact that the pHs of the release media seemed to have little effect on the synchronism of release behavior of the five components added evidence on drug properties-independent release mechanisms. However, pHs did affect the overall release rate with lower pH leading to reduced release rate with $P_{12}$ of about 55%, 65% and 90% at pH of 1.2, 4.0 and 6.5 (distilled water), respectively, showing enhanced sensitivity of the matrix to higher pH. As GMS had an ester

Table 3
Similarity factor $f_2$ values of each two of the release profiles from matrix system at different GMS/PEG6000 ratio

| GMS/PEG6000 ratio | TF/SC | TF/SD | TF/ISB | TF/SB | SC/SD | SC/ISB | SC/SB | SD/ISB | SD/SB | ISB/SB | $\overline{X} \pm SD$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5/95 | 54.68 | 68.65 | 53.17 | 54.30 | 66.06 | 98.30 | 97.68 | 64.37 | 65.67 | 94.96 | 71.78 ± 18.23 |
| 10/90 | 62.56 | 71.53 | 63.37 | 63.45 | 70.95 | 95.42 | 99.04 | 73.46 | 72.49 | 96.53 | 76.88 ± 14.47 |
| 15/85 | 75.63 | 82.82 | 82.95 | 87.98 | 78.44 | 88.30 | 85.37 | 83.21 | 90.54 | 92.52 | 84.78 ± 5.27 |
| 20/80 | 72.32 | 74.89 | 87.36 | 84.01 | 95.83 | 80.71 | 85.40 | 84.00 | 89.30 | 95.78 | 84.96 ± 7.75 |
| 30/70 | 74.61 | 77.07 | 82.07 | 81.51 | 88.88 | 80.07 | 86.36 | 89.89 | 94.91 | 95.21 | 85.06 ± 7.14 |
| 40/60 | 92.48 | 82.31 | 84.31 | 89.57 | 88.93 | 80.60 | 85.04 | 77.18 | 79.00 | 94.57 | 85.40 ± 5.84 |

Table 4
Similarity factor $f_2$ values of each two of the release profiles from matrix system at different GMS/PXM 188 ratio

| GMS/PXM 188 ratio | TF/SC | TF/SD | TF/ISB | TF/SB | SC/SD | SC/ISB | SC/SB | SD/ISB | SD/SB | ISB/SB | $\overline{X} \pm SD$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5/95 | 71.22 | 64.80 | 67.40 | 71.82 | 54.48 | 88.34 | 87.17 | 54.84 | 56.46 | 92.86 | 70.94 ± 14.29 |
| 7/93 | 71.22 | 70.76 | 68.05 | 69.65 | 61.27 | 83.83 | 87.44 | 63.20 | 63.46 | 97.99 | 73.69 ± 12.08 |
| 10/90 | 71.67 | 78.74 | 69.70 | 71.12 | 62.47 | 92.90 | 90.31 | 62.38 | 63.71 | 95.52 | 75.85 ± 12.83 |
| 20/80 | 75.60 | 86.61 | 72.99 | 75.35 | 68.74 | 95.62 | 97.81 | 67.59 | 68.81 | 95.26 | 80.44 ± 12.17 |
| 30/70 | 79.84 | 94.57 | 70.88 | 71.35 | 78.91 | 88.23 | 89.25 | 70.78 | 71.12 | 98.99 | 81.39 ± 10.70 |
| 40/60 | 83.09 | 96.42 | 71.74 | 74.38 | 85.89 | 85.96 | 90.64 | 73.82 | 76.57 | 97.00 | 83.55 ± 9.28 |

Table 5
Mean release percentage of taxifolin, silichrystin, silydianin, isosilybin and silybin at 12 h ($P_{12}$) from GMS/PEG6000 and GMS/PXM 188 systems

| GMS/carrier ratio | GMS/PEG6000 | GMS/PXM 188 |
|---|---|---|
| 5/95 | 92.21 ± 3.22 | 90.23 ± 5.19 |
| 7/93 | Not evaluated | 84.99 ± 4.32 |
| 10/90 | 87.72 ± 3.15 | 71.93 ± 3.49 |
| 15/85 | 65.92 ± 1.79 | Not evaluated |
| 20/80 | 52.77 ± 1.63 | 53.10 ± 2.58 |
| 30/70 | 50.46 ± 1.52 | 42.07 ± 2.21 |
| 40/60 | 40.87 ± 0.86 | 37.14 ± 2.20 |

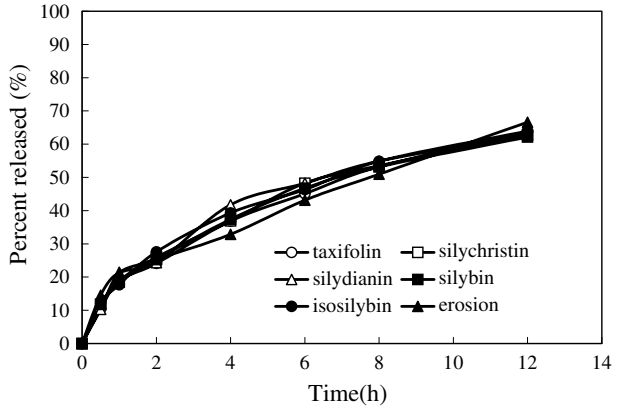


Fig. 7. Release profiles of multiple components of silymarin from and erosion of GMS/PEG6000 (15/85) matrix system with a drug/carrier ratio of 1/20 in pH 4.0 acetate buffer.

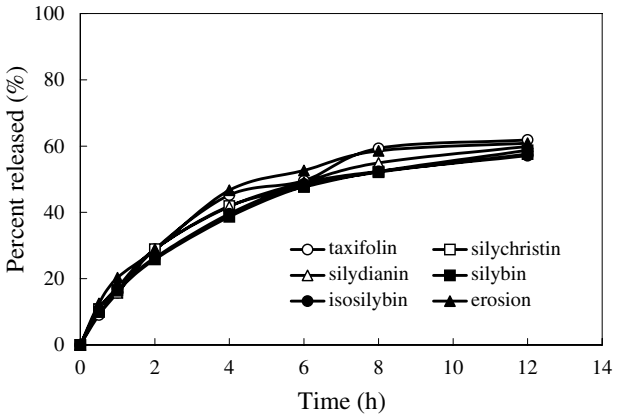


Fig. 8. Release profiles of multiple components of silymarin from and erosion of GMS/PEG6000 (15/85) matrix system with a drug/carrier ratio of 1/20 in pH 1.2 HCl solution.

Table 6
Release similarity factor $f_2$ values and overall release percentage at 12 h ($P_{12}$) at different drug/(GMS + PEG6000) ratios

| Silymarin/(GMS + PEG6000) | $f_2$[a] | $P_{12}$ (%)[b] |
|---|---|---|
| 1/10 | 85.58 ± 7.13 | 72.81 ± 1.75 |
| 1/20 | 84.78 ± 5.27 | 65.92 ± 1.79 |
| 1/30 | 76.37 ± 13.98 | 61.88 ± 4.09 |
| 1/40 | 70.82 ± 11.93 | 54.75 ± 5.68 |

[a] Mean ± SD of $f_2$ values between each two of the five components.
[b] Mean ± SD of the five components.

bond, its degradation speed was much higher at elevated pHs, which may lead to enhanced matrix erosion, hence drug release.

Silymarin content in the system also influenced synchronized release of the five components and overall release rate. Table 6 gives the mean of the similarity factor $f_2$ at different drug/(GMS + PEG6000) ratios from 1/10 to 1/40 and a GMS/PEG6000 ratio of 15/85. It was shown that within this drug/matrix range good similarity of the release profiles of the five components was observed, and there was a decreasing trend as the drug content kept decreasing. $P_{12}$ also decreased from 72.81% at component ratio of 1/10 to 54.75% at a ratio of 1/40. This served as another adjustable

factor that can be tuned to facilitate oral administration. Increase in drug content seemed to be beneficial to synchronism of drug release and helped to reduce the volume of the final product.

To decipher the mechanisms controlling synchronized release, erosion of GMS/PEG6000 matrix system were evaluated. In Figs. 6–8, erosion profiles was also provided and compared with release profiles. No significant difference ($P > 0.05$) was observed between erosion and release profiles at all release media at different pH. It is suggested that the release of the five components was totally erosion - controlled. There were exactly two types of erosion modes, i.e. surface erosion and bulk erosion [26,27]. For surface erosion, the water-penetrating speed was less than the erosion rate of the matrix, and erosion only took place at the surface. Release of drugs from surface erosion matrix was only a function of the erosion rate, and there was no contribution of diffusion to the final release. As for bulk erosion matrix, water-penetrating speed was faster than erosion speed and the matrix was quickly soaked by release medium. Contribution of diffusion to release in the latter erosion mode cannot be ignored. To elucidate the erosion mode of the GMS system, water uptake rate was evaluated. Fig. 9 shows that water uptake by the GMS/PEG 6000 (15/85) matrix system began at 1 h and was followed by a steady rise of water absorption in distilled water, pH 1.2 HCl solution and pH 4.0 acetate buffer, where there was approximately 226%, 310% and 338% relative water uptake at 12 h, respectively. The results showed that during the release process, there was a steady increase in water uptake, which did not support a mechanism of surface erosion. It was interesting that erosion-controlled release was obtained, although bulk erosion might be the predominant mechanism. Unlike systems of high porosity, which may be soaked quickly by water through the tortuous capillaries, the GMS/PEG or GMS/PXM matrix system did not possess capillaries, and hydration of the system only took place at the outer layer. Observed visually, the swelled layer looked more like that of swelled hydrogel like HPMC, and hydration only progressed gradually. Although water uptake percentage was high, there was virtually not much water in the matrix as the matrix had undergone profound degradation, which may result in high value of relative water uptake. Throughout the release process, the thickness of swelled layer of the matrix did not vary significantly which meant that the diffusion path was not long whether or not there was diffusion at all. In another hand, as all of the five components are relatively water-insoluble, their diffusivity in the swelled layer may be negligible, and the release might be completely erosion -controlled. However, there was still much work to be done to decipher the release mechanisms of these systems.

The purpose of strict controlling on synchronized release is to retain optimum and original activity of the mother herbal medicine as the release proceeds. Here, the effect of synchronized release on erythrocyte hemolysis by silymarin was studied and compared with samples of non-synchronized release. Fig. 10 shows the erythrocyte membrane protecting effect of synchronized release samples of silymarin that keep the original component ratio. The control group treated with blank saline shows the most significant hemolysis, and the hemolysis profile resides at utmost high NaCl concentration. As the concentration of silymarin, calculated as a whole of the

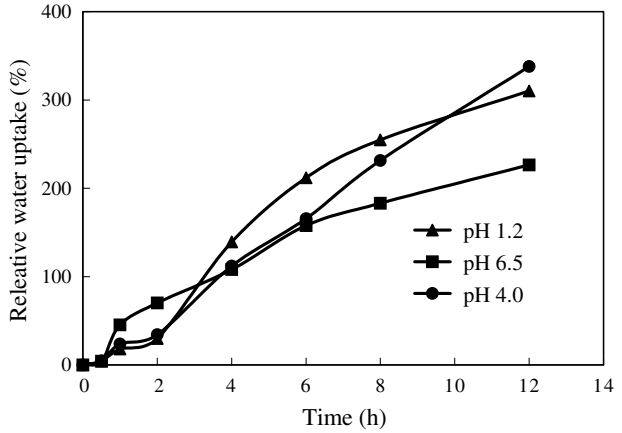


Fig. 9. Hydration of GMS/PEG 6000 (15/85) matrix system with a drug/carrier ratio of 1/20 in distilled water (pH 6.5), acetate buffer (pH 4.0) and 0.1 N HCl solution (pH 1.2).

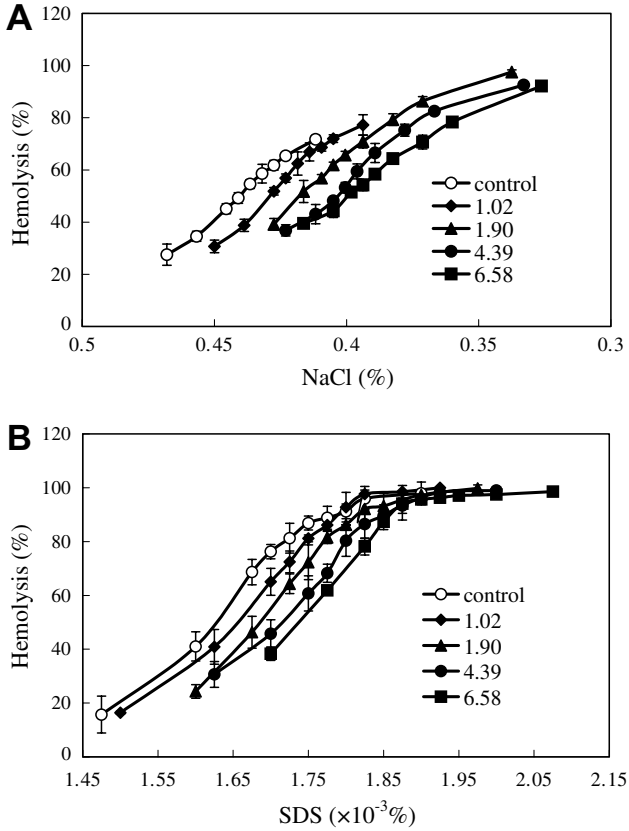


Fig. 10. Erythrocyte membrane-protecting effect of silymarin synchronized release samples at different total flavonoid levels (μg/ml) against osmotic (A) and SDS shock (B).

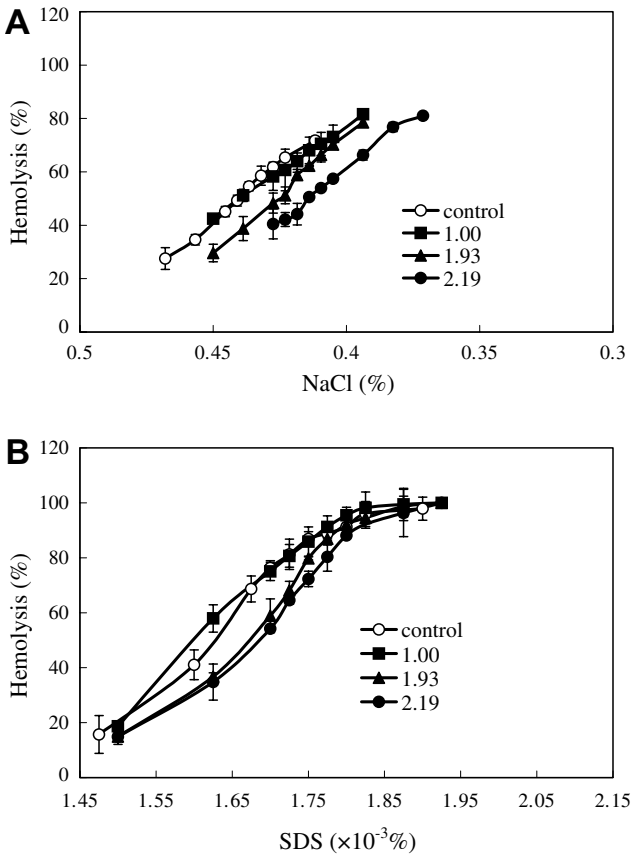


Fig. 11. Erythrocyte membrane-protecting effect of silymarin non-synchronized release samples at different total flavonoid levels (μg/ml) against osmotic (A) and SDS shock (B).

five flavonolignans, increased, the hemolysis profile moved left to the region of lower NaCl concentration, which meant that the ability of erythrocytes to withstand the osmotic shock increased with the aid of silymarin. There was a dose-dependent stabilization of the cell membrane. Similarly, the erythrocyte-protecting effect was observed in Fig. 10B. As silymarin concentration increased, there was a dose-dependent effect of withstanding the SDS shock. Fig. 11 shows the effect of non-synchronized release silymarin samples on erythrocyte hemolysis. It was obvious that at similar total flavonoid levels, the hemolysis profiles resided at a much lower level of shocking agent.

$H_{50}$s for synchronized and non-synchronized at different silymarin levels are given in Tables 7 and 8. ANOVA results indicated that there was a significant difference between synchronized release silymarin treated-group and control group under distilled water or SDS shock. However, for non-synchronized samples, there was no significant difference ($P > 0.05$) between the group treated with 1 μg/ml silymarin and the control group. Only at higher silymarin levels was there significant difference between silymarin and control group. Comparison between synchronized and non-synchronized release groups was also performed at the same level of total flavonoids. Results showed that at a level of about 1.00 and 1.90 μg/ml, the protecting effect of synchronized release samples was significantly better than that of non-synchronized release samples ($P < 0.05$). Comparison at higher levels has not been performed because dissolution of silymarin in isotonic

Table 7
50% hemolysis values ($H_{50}$) of synchronized release samples

| Total flavonoids (μg/ml) | Active components (μg/ml) | | | | | NaCl (%) with 95% confidence limits | SDS(×10$^{-3}$%) with 95% confidence limits |
|---|---|---|---|---|---|---|---|
| | TF | SC | SD | ISB | SB | | |
| Control | 0 | 0 | 0 | 0 | 0 | 0.44012 (0.43629, 0.44429) | 1.61055 (1.59358, 1.62523) |
| 1.02 | 0.03 | 0.22 | 0.06 | 0.14 | 0.57 | 0.42918* (0.42539, 0.43397) | 1.63081* (1.62264, 1.65147) |
| 1.90 | 0.05 | 0.41 | 0.11 | 0.27 | 1.06 | 0.41721* (0.41258, 0.42324) | 1.65541* (1.63885, 1.66920) |
| 4.39 | 0.12 | 0.95 | 0.26 | 0.61 | 2.45 | 0.40612* (0.40159, 0.41618) | 1.70241* (1.68686, 1.71561) |
| 6.58 | 0.18 | 1.42 | 0.39 | 0.92 | 3.67 | 0.40027* (0.39511, 0.40679) | 1.72985* (1.69530, 1.75440) |

* $P < 0.05$ vs. control.

Table 8
50% hemolysis values ($H_{50}$) of non-synchronized release samples

| Total flavonoids (μg/ml) | Active components (μg/ml) | | | | | NaCl (%) with 95% confidence limits | SDS (×10$^{-3}$%) with 95% confidence limits |
|---|---|---|---|---|---|---|---|
| | TF | SC | SD | ISB | SB | | |
| Control | 0 | 0 | 0 | 0 | 0 | 0.44012 (0.43629, 0.44429) | 1.61055 (1.59358, 1.62523) |
| 1.00 | 0.07 | 0.36 | 0.08 | 0.19 | 0.30 | 0.43903 (0.43312, 0.44837) | 1.61872 (1.59957, 1.63500) |
| 1.93 | 0.10 | 0.65 | 0.17 | 0.39 | 0.62 | 0.42398* (0.42014, 0.42838) | 1.66124*(1.63842, 1.68027) |
| 2.19 | 0.12 | 0.72 | 0.19 | 0.47 | 0.69 | 0.41394* (0.40975, 0.41908) | 1.67076* (1.65360, 1.68581) |

* $P < 0.05$ vs. control.

NaCl solution was limited, and it was difficult to obtain a higher concentration of total flavonoids of silymarin.

## 4. Conclusion

Development of sustained release delivery systems of herbal medicines demands strict controlling on synchronized release of multiple active components with a purpose to keep the original component ratios that defines the herbal medicines. Synchronized release of the five active components of silymarin (taxifolin, silychristin, silydianin, isosilybin and silybin) was achieved by an erodible matrix system made up of glyceryl monostearate and polyethylene glycol 6000 or poloxamer 188. The synchronism of their release profiles was characterized by similarity factor $f_2$ of over 60. Good correlation between erosion and release was also observed, indicating erosion-controlled release mechanisms. There was a gradual increase in relative water uptake, which supported bulk erosion rather than surface erosion mechanism. Increase of glyceryl monostearate content in the matrix led to higher $f_2$ values, but reduced release rate. Preliminary study on erythrocyte hemolysis showed that synchronized release samples were able to protect the erythrocyte more efficiently than non-synchronized release samples.

## References


[1] E.M. Williamson, Synergy and other interactions in phytomedicines, Phytomedicine 8 (2001) 401–409.

[2] X.H. Fan, Y.Y. Cheng, Z.L. Ye, R.C. Lin, Z.Z. Qian, Multiple chromatographic fingerprinting and its application to the quality control of herbal medicines, Anal. Chim. Acta 555 (2006) 217–224.

[3] B.Y. Li, Y. Hu, Y.Z. Liang, P.S. Xie, Y.P. Du, Quality evaluation of fingerprints of herbal medicine with chromatographic data, Anal. Chim. Acta 514 (2004) 69–78.

[4] Y.Z. Liang, P.S. Xie, K. Chan, Quality control of herbal medicines, J. Chromatogr. B 812 (2004) 53–70.

[5] M.F. Qiu, W. Jia, S.S. Li, Z.H. Xu, X. Sun, X.R. Wang, Y.Y. Zhang, G.X. Xie, A new silymarin preparation based on solid dispersion technique, Adv. Ther. 22 (2005) 595–600.

[6] H.T. Song, T. Guo, R.H. Zhang, C.L. Zheng, Y. Ma, X. Li, K.S. Bi, X. Tang, Preparation of the traditional chinese medicine compound recipe heart-protecting musk pH-dependent gradient-release pellets, Drug Dev. Ind. Pharm. 28 (2002) 1261–1273.

[7] M. Baluom, M. Friedman, A. Rubinstein, Improved intestinal absorption of sulpiride in rats with synchronized oral delivery systems, J. Control. Release 70 (2001) 139–147.

[8] K.K. Peh, C.F. Wong, K.H. Yuen, Possible mechanism for drug retardation from glyceryl monostearate matrix system, Drug Dev. Ind. Pharm. 26 (2000) 447–450.

[9] C.M. Adeyeye, J.C. Price, Development and evaluation of sustained-release ibuprofen-wax microspheres. I. Effect of formulation variables on physical characteristics, Pharm. Res. 8 (1991) 1377–1383.

[10] C.M. Adeyeye, J.C. Price, Development and evaluation of sustained-release ibuprofen-wax microspheres. II. In vitro dissolution studies, Pharm. Res. 11 (1994) 575–579.

[11] S. Allababidi, J.C. Shah, Kinetics and mechanism of release from glyceryl monosterate-based implants: evaluation of release in a gel simulating in vivo implantation, J. Pharm. Sci. 87 (1998) 738–744.

[12] S. Allababidi, J.C. Shah, Efficacy and pharmacokinetics of site-specific cefazolin delivery using biodegradable implants in the prevention of post-operative wound infections, Pharm. Res. 15 (1998) 325–333.

[13] Y. Chen, G.G. Zhang, J. Neilly, K. Marsh, D. Mawhinney, Y.D. Sanzgiri, Enhancing the bioavailability of ABT-963 using solid dispersion containing Pluronic F-68, Int. J. Pharm. 286 (2004) 69–80.

[14] D. Law, W. Wang, E.A. Schmitt, Y. Qiu, S.L. Krill, J.J. Fort, Properties of rapidly dissolving eutectic mixtures of poly(ethylene glycol) and fenofibrate: the eutectic microstructure, J. Pharm. Sci. 92 (2003) 505–515.

[15] J. Pepping, Milk thistle: Silybum marianum, Am. J. Health Syst. Pharm. 56 (1999) 1195–1197.

[16] T. Ding, S. Tian, Z. Zhang, D. Gu, Y. Chen, Y. Shi, Z. Sun, Determination of active components in silymarin by RP-LC and LC/MS, J. Pharm. Biomed. Anal. 26 (2001) 155–161.

[17] F. Kvasnička, B. Bíba, R. Ševčík, M. Voldřich, J. Kráthká, Analysis of the active components of silymarin, J. Chromatogr. A 990 (2003) 239–245.

[18] J.W. Moore, H.H. Flanner, Mathematical comparison of curves with an emphasis on in vitro dissolution profiles, Pharm. Tech. 20 (1996) 64–74.

[19] V. Pillay, R. Fassihi, Evaluation and comparison of dissolution data derived from different modified release dosage forms: an alternative method, J. Control. Release 55 (1998) 45–55.

[20] J.E. Polli, G.S. Rekhi, L.L. Augsburger, V.P. Shah, Methods to compare dissolution profiles and a rationale for wide dissolution specifications for metoprolol tartrate tablets, J. Pharm. Sci. 86 (1997) 690–700.

[21] S. Cafaggi, R. Leardi, B. Parodi, G. Caviglioli, E. Russo, G. Bignardi, Preparation and evaluation of a chitosan salt-poloxamer 407 based matrix for buccal delivery, J. Control. Release 102 (2005) 159–169.

[22] N.K. Ebube, A.B. Jones, Sustained release of acetaminophen from a heterogeneous mixture of two hydrophilic non-ionic cellulose ether polymers, Int. J. Pharm. 272 (2004) 19–27.

[23] A. Valenzuela, T. Barria, R. Guerra, A. Garrido, Inhibitory effect of the flavonoid silymarin on the erythrocyte hemolysis induced by phenylhydrazine, Biochem. Biophy. Res. Comm. 126 (1985) 712–718.

[24] F. Wang, T. Wang, J. Lai, M. Li, C. Zou, Vitamin E inhibits hemolysis induced by hemin as a membrane stabilizer, Biochem. Pharmacol. 71 (2006) 799–805.

[25] S. Shalel, S. Streichman, A. Marmur, Monitoring surfactant-induced hemolysis by surface tension measurement, J. Colloid Interf. Sci. 255 (2002) 265–269.

[26] F. Burkersroda, L. Schedl, A. Göpferich, Why degradable polymers undergo surface erosion or bulk erosion, Biomaterial 23 (2002) 4221–4231.

[27] S.Y. Ng, H.R. Shen, E. Lopez, Y. Zherebin, J. Barr, E. Schacht, J. Heller, Development of a poly(ortho ester) prototype with a latent acid in the polymer backbone for 5-fluorouracil delivery, J. Control. Release 65 (2000) 367–374.